Serum immunoglobulin heavy/light chain (HLC) and free light chain (FLC) concentrations at diagnosis in multiple myeloma and IgM malignant lymphoma patients with survival exceeding 10 years

Stężenia w surowicy wolnych lekkich łańcuchów immunoglobulinowych (FLC) i łańcuchów lekkich związanych z łańcuchem ciężkim (HLC), w chwili rozpoznania choroby u chorych na szpiczaka plazmocytowego i chłoniaka złośliwego IgM z czasem przeżycia ponad 10 lat

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SUMMARY

Immunoglobulin (Ig) heavy/light chain (HLC) is a novel antibody based assay that separately measures in pairs the light chain types of each Ig class generating ratios of monoclonal Ig/background polyclonal Ig concentrations. Free light chain (FLC) assay measures unbound κ and λ chains. The aim of the present study was to assess the prognostic impact of HLC and FLC assays in multiple myeloma (MM) and IgM malignant lymphoma (ML) patients with real long term survival. Measurements of serum HLC and FLC concentrations were performed in 23 MM and 12 ML patients with survival exceeding 10 years and 43 (19 MM, 24 ML) patients with survival not exceeding 5 years. HLC and FLC ratios at diagnosis were less abnormal in patients with survival exceeding 10 years than in patients with survival up to 5 years (p=0.03). The differences in median values were manifold. However, in patients with survival over 10 years highly abnormal HLC ratio (<0.022 or >45) was found in 3 MM patients and 7 ML patients and highly abnormal FLC ratio (<0.1 or >30) was found in 5 MM patients and in 1 ML patient. In conclusion, serum HLC and FLC measurements at MM diagnosis provide prognostic information, despite that even in MM patients with survival exceeding 10 years in 15% of them at diagnosis serum HLC and FLC ratios may be highly abnormal.

Key words: Heavy/light chain assay, Free light chains, Multiple myeloma, Waldenström macroglobulinemia, Survival

STRESZCZENIE

Opracowany ostatnio immunoglobulinowy test „Hevylite" HLC (*heavy/light chain*) pozwala na osobne mierzenie typów łańcuchów lekkich w parach każdej klasy immunoglobulinowej i oznaczanie stosunku stężenia monoklonalnej immunoglobuliny do stężenia poliklonalnej immunoglobuliny tej samej klasy. Test FLC (*free light chain*) oznacza wolne lekkie łańcuchy κ i λ, niezwiązane z cząsteczką immunoglobulinową. Celem aktualnych badań była ocena wartości prognostycznej oznaczania w chwili rozpoznania choroby HLC i FLC w surowicy chorych na szpiczaka plazmocytowego i chłoniaka złośliwego IgM z rzeczywiście długim czasem przeżycia. Wykonano oznaczenia stężenia w surowicy HLC i FLC u 23 chorych na szpiczaka i 12 na chłoniaka z czasem przeżycia ponad 10 lat oraz u 43 chorych (19 na szpiczaka, 24 na chłoniaka) z czasem przeżycia nieprzekraczającym 5 lat. Wartości stosunku HLC i stosunku FLC w chwili rozpoznania choroby były w mniejszym stopniu nieprawidłowe u chorych z czasem przeżycia ponad 10 lat niż u chorych z czasem przeżycia poniżej 5 lat (p=0,03). Różnica w wartościach średnich była wielokrotna. Jednakże u chorych z czasem przeżycia ponad 10 lat wysoce nieprawidłowe wartości stosunku HLC (<0,022 lub >45) stwierdzono u 3 chorych na szpiczaka plazmocytowego i 7 chorych na chłoniaka IgM, a wy-

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soce nieprawidłowe wartości stosunku FLC (<0,1 lub >30) stwierdzono u 5 chorych na szpiczaka i 1 chorego na chłoniaka. Z badań wynika, że oznaczanie HLC i FLC w czasie rozpoznania szpiczaka dostarcza informacji prognostycznych, mimo że nawet u 15% chorych z czasem przeżycia ponad 10 lat w chwili rozpoznania wartości stosunku HLC i stosunku FLC w surowicy mogą być wysoce nieprawidłowe.

Słowa kluczowe: Hevylite, wolne łańcuchy lekkie, szpiczak plazmocytowy, makroglobulinemia Waldenströma, przeżycia

Introduction

Recently, an assay for serum immunoglobulin free light chains (FLCs) has become available for clinical use [1] and is currently applied to monitor patients with plasma cell disorders [2–4]. The assay allows quantitation of kappa and lambda chains that are not bound to intact immunoglobulin molecules, and allows determination of clonality based on the kappa to lambda ratio. An abnormal FLC ratio indicating presence of monoclonal free light chains has been recently shown to be an independent risk factor for progression in monoclonal gammopathy of undetermined significance (MGUS) and for progression of smoldering (asymptomatic) multiple myeloma (MM) [5, 6] and solitary plasmacytoma of bone [7]. Snozek et al. [8] and Kyrtsonis et al. [9, 10] suggest that the serum FLC ratio at initial diagnosis is an important predictor of prognosis in myeloma and can be incorporated into the International Staging System [11] for improved risk stratification.

In 2009 Bradwell et al. [12] developed immunoglobulin heavy/light chain immunoassays – "Hevylite" (HLC). Availability of antibodies which bind to conformational epitopes spanning the junctional regions between bound κ or λ light chains and their respective heavy chain partners has allowed the specific measurement of serum IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ concentrations. In turn, this has enabled the calculation of IgGκ/IgGλ, IgAκ/IgAλ and IgMκ/IgMλ ratios (heavy/light chain or HLC ratios) for individual patients. Separate measurements of the κ and λ light chain types of IgG, IgA and IgM allow evaluation of individual tumor clones and give quantitative information about the immunosuppression of each non-tumor immunoglobulin. Measurement of molecule pairs, such as IgGκ/IgGλ, IgAκ/IgAλ, IgMκ/IgMλ, would indicate clonality in the same manner as serum free light chain (FLC) κ/λ ratios [1].

Until now, apart from a key Bradwell's et al. [12] and our [13] publications, and case report [14], there appeared congress reports on the results of the use of nephelometric measurement of individual immunoglobulin κ/λ ratios in assessment of monoclonal gammopathies including evaluation of prognostic value of Hevylite assays in these diseases [15–21]. The aim of the present study was to assess the prognostic impact of FLC and HLC assays in MM and IgM malignant lymphoma (ML) patients with real long term – 10 years or more – survival.

Material and methods

The study included 23 MM and 12 ML patients with survival exceeding 10 years and 43 (19 MM, 24 ML) patients with survival not exceeding 5 years. All patients were diagnosed [22] and follow-up for many years at the Institute of Hematology and Transfusion Medicine in Warsaw.

Serum protein electrophoresis and immunofixation were performed on agarose media with densitometric scanning using Hydrasys™ 2 apparatus (Sebia, France) and antisera from the same company and also using Beckman Paragon Immunofixation Electrophoresis Kit.

Nephelometric immunoglobulin assays, performed using a Siemens BN™ II nephelometer, were used to measure IgGκ/IgGλ, IgAκ/IgAλ, IgMκ/IgMλ (HLC) and also to quantify FLC in the archived frozen and fresh sera of assessed patients. In this method there were applied antibodies (Hevylite™ Human IgG Kappa Kit; IgG lambda Kit, Hevylite™ Human IgA Kappa Kit; IgA Lambda Kit, Hevylite™ Human IgM Kappa Kit; IgM Lambda Kit; The Binding Site, Ltd, Birmingham, UK) specific for IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, IgMλ and antibodies (Freelite®; The Binding Site, Ltd, Birmingham, UK) specific for κ and λ light chains in free form, not bound to the heavy chain.

Statistical analysis was performed using Fisher exact test.

Results

Clinical and immunoglobulin study at diagnosis in individual MM and ML patients with survival exceeding 10 years are presented in tables I–III. Table IV presents serum FLC and HLC IgG concentrations in a patient with POEMS syndrome and survival exceeding 9 years.

Table V summarizes results of tumor and non-tumor HLC immunoglobulins concentrations and HLC

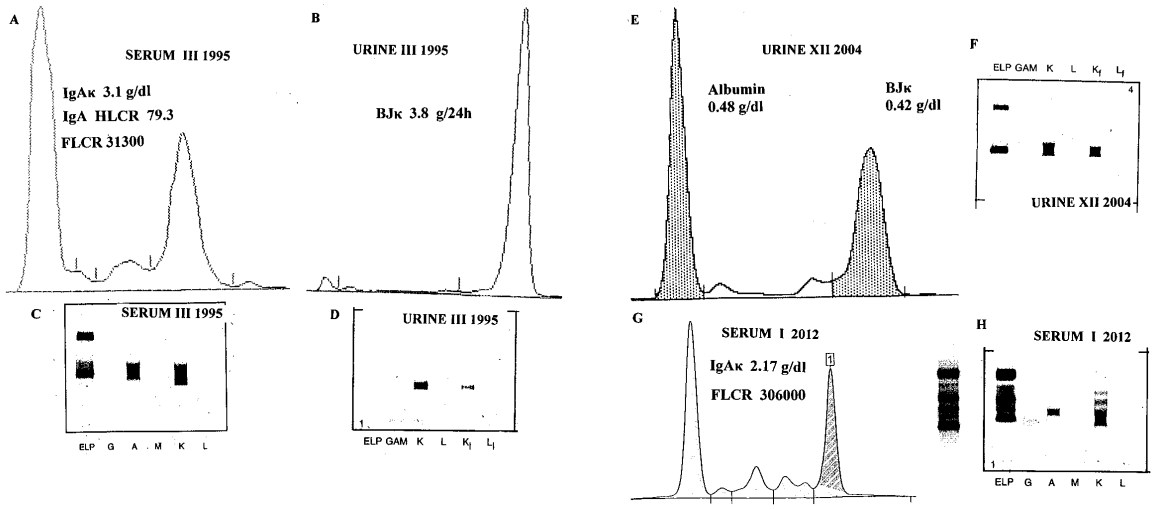

Fig. 1. Enormously high values of serum FLC ratios in a patient with IgAκ multiple myeloma and renal failure, at diagnosis (A) and 17 years later at relapse (G)
Ryc. 1. Niezwykle wysokie wartości stosunku wolnych łańcuchów lekkich w surowicy chorego na szpiczaka plazmocytowego IgAκ i z niewydolnością nerek, w chwili rozpoznania choroby (A) i 17 lat później w czasie progresji choroby (G)

IgGκ/IgGλ, IgAκ/IgAλ, IgMκ/IgMλ ratios at diagnosis in sera of patients with IgG and IgA MM and IgM ML with survival exceeding 10 years and table VI summarizes results of that study in patients with survival not exceeding 5 years. Table VII presents serum FLC concentrations at diagnosis in patients with MM and ML with survival exceeding 10 years and table VIII presents results of that study in patients with survival not exceeding 5 years. Fig. 1 illustrates serum and urine protein study results in a still living patient with MM and acute renal failure at diagnosis and during 17 years period of observation.

Discussion

In our previous study out of 600 assessed patients with MM 45 (7.5%) survived over 10 years from the disease diagnosis and beginning of antitumor treatment. Patients with long survival were younger (median, age 55 years) at the time of diagnosis than the whole studied group and had normal serum creatinine, calcium and β_2M levels. Sixty eight percent of these patients had stage I or II clinical progression [23]. Also in present study MM patients with survival exceeding 10 years had following characteristics at diagnosis: median age 52 years (range 31–78), median serum β_2M concentration 2.46 mg/l (range 1.24–4.78), median serum monoclonal component concentration 3.1 g/dl (range 1.24–4.78); 70% of these patients had stage I disease according to International Staging System criteria [11] (Tab. I–III). Present study add to these characteristics only slightly to moderately, in comparison to the general MM population, abnormal HLC and FLC ratios (Tab. VII–IX).

When patients were stratified according to their survival time over 10 years or not exceeding 5 years serum HLC and FLC ratios at diagnosis were less abnormal in patients with survival exceeding 10 years (p=0.03). The differences in median values were manifold (Tab. VII–IX). However, in patients with survival exceeding 10 years highly abnormal HLC ratio (<0.022 or >45) was found in 3 MM patients (13.6%) and 7 ML patients (58%) and highly abnormal FLC ratio (<0.1 or >30) was found in 5 MM patients (23.8%) and in 1 ML patient (8%). Manier et al. [21] conducted the study in a series of 71 Waldenström Macroglobulinemia patients at diagnosis. The median serum IgM HLC ratio was 100 (2.59–2850) and the median IgM involved HLC level was 21.9 (1.94–126) g/l. It is worthy to notice that our still living patient with MM and renal failure had at diagnosis and have also presently, 17 years later, enormously increased serum FLC ratio (Fig. 1).

Previously we reported [25] the case of a male patient presenting with polyneuropathy, IgGλ monoclonal gammopathy, splenomegaly, inconsiderable lymphadenopathy, disseminated sclerotic and single lytic bone lesions, papilledema, leg edemas, discrete hypothyroidism and thrombocytosis. The POEMS syndrome was diagnosed and the patient was initially treated with the VAD regimen. No improvement of neuropathy was observed, but the other disease symptoms did not progress. One year after diagnosis the patient underwent myeloablative treatment, followed by autologous peripheral blood stem cell transplantation. Subjective neurological amelioration, normalization of the platelet count and stabi-

Table I. Serum free light chain (FLC) and heavy /light chain (HLC) IgG concentrations at diagnosis in individual IgG multiple myeloma patients and survival exceeding 10 years.

Tabela I. Stężenia wolnych łańcuchów lekkich (FLC) i związanych z łańcuchem ciężkim (HLC) IgG w chwili rozpoznania choroby u chorych na szpiczaka plazmocytowego IgG z czasem przeżycia ponad 10 lat

Case	Age (yr)	Date of serum sample	Serum β_2M (mg/l)	ISS stage	Isotype of M-protein	Serum M-protein (g/dl)	HLC IgG g/l		HLC ratio IgGκ/ IgGλ	κ FLC (mg/l)	λ FLC (mg/l)	FLC ratio κ/λ	Survival time (mo)	Present status
							IgGκ	IgGλ						
1.	52	IV 1987	1.27	I	IgGλ	3.2	9.83	27.80	0.35	6.31	95.60	0.07	120	died
2.	78	X 1991	2.65	I	IgGλ	3.1	<1.69	25.60	< 0.06	13.40	421.00	0.03	114	died
3.	52	XI 1987	1.36	I	IgGκ	3.1	37.50	1.61	23.40	99.70	11.60	8.56	197	died
4.	47	VII 1999 VIII 2008 XI 2011	2.46	I	IgGκ	6.3	97.60 11.80 7.41	1.95 3.62 3.22	50.20 3.27 2.30	143.00 13.60 6.80	12.70 9.38 7.25	11.20 1.45 0.94	> 192	CR
5.	52	V 1981	2.88	I	IgGκ	9.7	63.70	10.20	6.25	59.00	25.20	2.35	264	died
6.	60	VIII 2000	2.24	I	IgGκ	3.5	38.60	0.66	58.50	55.30	6.27	8.81	128	died
7.	53	I 1985 III 2011	2.56	I	IgGκ	2.6	35.50 25.70	3.41 1.53	10.40 16.70	60.10	21.50	2.80	> 312	SD
8.	50	XII 1991	4.78	II	IgGλ	8.7	6.80	108.00	0.06	6.75	56.90	0.12	145	died
9.	44	I 1997 IV 2010 V 2011	1.90	I	IgGλ	3.0	2.23 1.23 0.67	25.60 23.30 31.80	0.09 0.05 0.02	14.40	116.00	0.12	>180	SD
10.	64	VII 1993	3.47	II	IgGκ	3.3	11.60	1.46	7.94	46.00	5.63	8.18	123	died
11.	41	I 1995 II 2005 VI 2005	1.84	II	IgGκ	1.8	12.10 5.18	1.02 0.41	11.80 12.60	592.00 7800 6210	5.33 1.26 0.28	99.80 6170 21700	172	died
12.	60	VIII 2003	5.11	II	IgGλ	6.0	3.62	52.20	0.07	1.56	2240	0.00…	> 108	PR

Abbreviations: **CR** – complete response, **PR** – partial response, **SD** – stabilisation of disease

Table II. Serum free light chain (FLC) and heavy /light chain (HLC) IgA concentrations at diagnosis in individual IgA multiple myeloma patients with survival exceeding 10 years

Tabela II. Stężenia wolnych łańcuchów lekkich (FLC) i związanych z łańcuchem ciężkim (HLC) IgA w chwili rozpoznania choroby u chorych na szpiczaka plazmocytowego IgA z czasem przeżycia ponad 10 lat

Case	Age (yr)	Date of serum sample	Serum β_2M (mg/l)	ISS stage	Isotype of M-protein	Serum M-protein (g/dl)	HLC IgA g/l		HLC ratio IgAκ/ IgAλ	κ FLC (mg/l)	λ FLC (mg/l)	FLC ratio κ/λ	Survival time (mo)	Present status
							IgAκ	IgAλ						
1.	55	I 1986	3.89	II	IgAλ	3.15	10.40	65.80	0.16	6.76	26.30	0.26	117	died
2.	69	VI 1992 VII 1995	1.81	I	IgAκ	3.00 3.80	38.80 42.20	1.64 0.68	23.60 61.60	24.20 22.60	5.84 5.43	4.14 4.16	> 120	SD
3.	57	III 1998	1.43	I	IgAκ	2.90	25.10	3.48	7.22	55.90	7.85	7.12	130	died
4.	32	III 2001 VIII 2011	1.24	I	IgAλ	2.10	3.13	35.80	0.09	6.40 1.45	71.70 1.88	0.09 0.78	> 130	CR
5.	71	III 1996	2.25	I	IgAκ	1.20	18.10	0.86	20.90	42.50	11.60	3.68	> 190	SD
6.	57	VI 2001 I 2012	2.72	I	IgAκ	4.77	51.00	1.50	34.00	26.10 10.00	5.91 2.53	4.42 3.96	> 127	nCR
7.	41	XII 1989	1.32	I	IgAκ	2.38	2.42	0.47	5.13	16.50	11.40	1.45	> 168	SD
8.	31	VIII 2002 II 2011	3.74	III	IgAκ	6.41	80.10	9.40	8.53	12.10 47.50	< 0.26 7.26	> 46 6.54	>113	PR
9.	43	III 1995 I 2012	8.85	II	IgAκ	3.10 2.17	43.20 2.81	0.54 0.06	73.30	9380 126000	0.30 0.41	31300 306000	> 202	PD
10.	63	I 1998			IgAκ	0.26	10.30	0.17	43.00				> 156	PR

Abbreviations: **SD** – stabilisation of disease, **CR** – complete response, **PR** – partial response, **PD** – progression of disease, **mo** – month

Table III. Serum free light chain (FLC) and heavy /light chain (HLC) IgM concentrations at the time of diagnosis in individual patients with IgM malignant lymphoma and survival exceeding 10 years

Tabela III. Stężenia wolnych łańcuchów lekkich (FLC) i związanych z łańcuchem ciężkim (HLC) IgM w chwili rozpoznania choroby u chorych z chłoniakiem złośliwym IgM z czasem przeżycia ponad 10 lat

Case	Age (yr)	M-protein (g/dl)		HLC IgM g/l		HLC ratio IgMκ/ IgMλ	κ FLC (mg/l)	λ FLC (mg/l)	FLC ratio κ/λ	Survival time (mo)
				IgMκ	IgMλ					
1.	37	IgMκ	2.72	24.30	2.72	8.93	37.30	13.50	2.76	192
2.	62	IgMκ	4.43	99.70	0.03	2680	79.50	5.35	14.90	156
3.	60	IgMκ	2.20	123.00	1.91	64.20	3450	312	11.00	>168
4.	41	IgMλ	1.63	1.55	29.40	0.05	24.00	39.40	0.61	>120
5.	46	IgMλ	2.40	0.91	62.60	0.01	10.20	123.00	0.08	>120
6.	76	IgMκ	1.10	20.20	0.19	104.00	380	13.20	28.80	>228
7.	47	IgMκ	1.46	2.09	0.25	8.20	26.40	9.45	2.80	>132
8.	67	IgMκ	1.88	35.20	0.06	588.0	142.00	9.08	15.70	>132
9.	60	IgMλ	2.95	0.77	19.60	0.04	12.30	95.60	0.13	120
10.	44	IgMκ	1.20	11.10	2.63	4.22	9.49	15.80	0.60	>120
11.	63	IgMκ	2.70	18.30	0.09	192.0	22.00	11.00	2.00	>132
12.	70	IgMκ	5.20	353.00	0.50	698.0	115.0	6.79	16.90	>108

Table IV. Serum free light chain (FLC) and heavy /light chain (HLC) IgG concentrations at diagnosis and in the period of follow-up in patient with POEMS syndrome and survival exceeding 9 years

Tabela IV. Stężenia wolnych łańcuchów lekkich (FLC) i związanych z łańcuchem ciężkim (HLC) IgG w chwili rozpoznania choroby i przebiegu obserwacji w surowicy chorego z zespołem POEMS i czasem przeżycia ponad 9 lat

Date of serum sample	Serum β_2M (mg/l)	ISS stage	M-protein by IFE and SPE (g/dl)	HLC IgG g/l		HLC ratio IgGκ/ /IgGλ	κ FLC (mg/l)	λ FLC (mg/l)	FLC ratio κ/λ
				IgGκ	IgGλ				
V 2003 (at diagnosis)	2.17	I	IgG λ 0.80	6.60	8.20	0.80	47.10	73.30	0.64
III 2005 (after ASCT)				6.01	7.31	0.82			
X 2011 (at follow-up)				3.82	6.85	0.56	24.10	50.60	0.47

Abbreviations: **ASCT** – autologous stem cell transplantation

lization of other symptoms was observed. Since that time for 8 years the patient was followed without further treatment. Stabilization of the disease was also reflected in stable serum immunoglobulin picture: slightly abnormal IgGκ/IgGλ HLC ratio, normal uninvolved IgGκ HLC concentration and normal FLC ratio (Tab. IV).

Some congress publications provide data on prognostic value of HLC analysis in multiple myeloma and MGUS [16–21].

Avet-Loiseau et al. [16, 17] reported that heavy/light chain specific immunoglobulin ratios at presentation were prognostic for progression free survival (PFS) in the IFM 2005-01 myeloma trial. In that study, Kaplan Meier analysis indicated that more abnormal HLC ratios were associated with reduced PFS (> median for IgGκ and IgAκ patients, < median for IgGλ and IgAλ patients; p=0.007). When using more extreme ratios (>200 or <0.01), the significance level was higher increased (p=0.002). Cox regression analysis confirmed the association of the latter HLC ratios with reduced PFS (p<0.001) and indicated that the association was independent of and more significant than that of β_2M or albumin. The combined use of the extreme HLC ratios and β_2M >3.5 mg/L, in a risk stratification model, showed significant differences in PFS for patients with 0, 1, or 2 adverse risk factors (p=0.000013). A more complex risk stratification model combining HLC ratios with the International Staging System also showed significant differences in PFS according to the number of risk factors (p=0.0001). The use of HLC ratios provides a measure of both tumor immunoglobulin production and immunoparesis. Probably, the combination of these two factors has a prognostic value. HLC measurements may be a useful addition to the current ISS assessments.

Also Ludwig et al. [19, 20] found that the ratio of monoclonal to polyclonal immunoglobulins assessed

Table V. Tumor and non-tumor HLC concentrations and HLC IgGκ/IgGλ, IgAκ/IgAλ, IgMκ/IgMλ ratios at diagnosis in sera of patients with IgG and IgA multiple myeloma and IgM malignant lymphoma with survival exceeding 10 years

Tabela V. Stężenia nowotworowej i nie nowotworowej HLC i wartości stosunku IgGκ/IgGλ, IgAκ/IgAλ, IgMκ/IgMλ HLC w chwili rozpoznania choroby w surowicach chorych na szpiczaka plazmocytowego IgG i IgA oraz chłoniaka złośliwego IgM z czasem przeżycia ponad 10 lat

Tumor sera with M-protein isotype acc. to IFE	n	HLC immunoglobulin g/L		HLC ratios IgGκ/IgGλ	n	HLC immunoglobulin g/L		HLC ratios IgAκ/IgAλ	n	HLC immunoglobulin g/L		HLC ratios IgMκ/IgMλ
		IgGκ x±SD Median, range	IgGλ x±SD Median, range	x±SD Median, range		IgAκ x±SD Median, range	IgAλ x±SD Median, range	x±SD Median, range		IgMκ x±SD Median, range	IgMλ x±SD Median, range	x±SD Median, range
MM IgGκ	7	42.37±30.14 37.50 11.60–97.60	2.90±3.33 1.61 0.66–10.20	24.0±21.5 11.80 7.94–58.50								
MM IgGλ	6	5.13±3.87 4.51 1.69–9.83	46.75±40.81 26.7 25.6–108	0.14±0.14 0.09 0.06–0.35								
MM IgAκ					8	37.44±25.2 42.2 2.42–80.1	2.41±3.25 0.86 0.54–9.40	30.95±29.21 20.90 5.13–79.30				
MM IgAλ					2	10.40; 3.13	65.80 35.80	0.09; 0.16				
ML IgMκ									9	76.30±111.7 24.3 2.09–353	0.93±1.14 0.25 0.03–2.72	483.0±863 104.00 4.22–2680.00
ML IgMλ									3	1.07±0.41 0.91 0.77–1.55	37.2±22.5 29.4 19.6–62.6	0.03±0.02 0.04 0.01–0.05
Blood donor sera (acc. to Bradwell et al. [12].	109	7.76 4.23–12.18	4.00 2.37–5.91	1.96 1.26–3.2	191	1.27 0.43–2.36	0.87 0.4–1.73	1.4 0.58–2.52	118	0.77 0.33–1.54	0.50 0.20–1.10	1.6 0.81–2.52

Abbreviations: **HLC** – heavy chain/ light chain; **IFE** – immunofixation electrophoresis; **MM** – multiple myeloma; **ML** – malignant lymphoma

with the Hevylite test predicts prognosis, is superior for monitoring the course of the disease and allows detection of monoclonal immunoglobulin in multiple myeloma patients with normal or subnormal involved immunoglobulin isotype. In a study of 103 multiple myeloma patients median overall survival of the entire group was 37.9 months. In multivariate analysis, β_2M, and HLC ratio were found as the only parameters correlating with survival. A three tiered risk stratification model utilizing β_2M >3.5 mg/L, and HLC > median value had a grater prognostic value than ISS (p=0.001 *vs* p=0.09). Patients with 0 risk factors (β_2M <3.5 mg/L, HLC ratio < median) had a 50% survival time of 118 months, patients with 1 risk factor (either β_2M >3.5 mg/L or HLC ratio > median) had a 50% survival of 53 months and those with both risk factors (β_2M >3.5 mg/L and HLC ratio > median) had a 50% survival of 29 months (p=0.001) [19]. In recent analysis when patients were stratified according to their presentation HLC ratios being moderately abnormal (0.022–45; n=51) or highly abnormal (<0.022 or >45; n=52), survival was significantly shorter in those with highly abnormal ratios (median 32,1 months versus median not reached, p=0.016). The survival rates at 5 years were 33.4% for the former and 58.9% for the latter group (p=0.01). For patients with a highly abnormal FLC ratio (<0.1 or >30) a statistically non-significant tendency for shorter survival was noted (40.8 months versus median not reached, p=0.08) compared to those with less abnormal FLC ratios (0.1–30). A risk stratification prognostic model with highly abnormal HLC and FLC ratios as risk factors at presentation was developed. Overall survival was significantly different between patients with both, highly abnormal HLC and FLC ratios or only one, or none of these risk factors (p=0.01). The median was not reached in patients with 0 or 1 risk factor and was 29.2 months in those with 2 risk factors. The respective five year survival rates were 67.4%, 50.0% and 23.3% [20].

The serum FLC ratio at presentation has been shown to be an independent prognostic marker in multiple myeloma [8]. Also Harding et al. [15] reported the use of serum FLC and HLC ratios to predict survival in multiple myeloma patients. Archived presentation sera (n=186) from British Medical Research Council multiple myeloma trials were analyzed retrospectively using sFLC and HLC assays. Kaplan-Meier survival curves were constructed to compare patients above or below the median value for FLC and HLC ratio and M-spike concentration. There was no significant difference in survival when analyzing intact M protein concentrations, serum FLC concentrations, FLC ratios or HLC ratios individually. Adding FLC and HLC ratios together there was a significantly shorter survival for those patients with values greater then

the median (50% survival 949 days versus 1592 days; p=0.02). The serum FLC ratio is likely to be more predictive of outcome than the concentration of the tumor sFLC because it includes a measure of immunoparesis (the denominator) as well as tumor production (the numerator). Use of the HLC ratio also incorporates a measure of immunoparesis but additionally, it will compensate for any reduction in the concentration of monoclonal immunoglobulin due to increased catabolism (IgG) and/or increased plasma volume. It is probable that the use of the summated sFLC and HLC ratios was more predictive of outcome because there were some patients with very low monoclonal intact immunoglobulin production for whom the FLC ratio was the most appropriate prognostic marker and *vice versa* for patients with low FLC production [15].

A carried out in our previous study [13] evaluation of actual survival time of 21 IgM gammopathy patients diagnosed with multiple myeloma or Waldenström's macroglobulinemia and mean follow-up time of six years showed no statistically significant difference in median survival depending on the results of the HLC ratios tested at diagnosis amounting 7 years in patients with HLC ratio values < the median and 5.5 years for patients with values of HLC ratio > the median (p=0.2738). In the present study highly abnormal HLC ratio (<0.022 or >45) was found in 7 out of 12 (58%) IgM ML patients with survival exceeding 10 years.

However, Koulieris et al. [26] evaluated the prognostic value of IgMκ/IgMλ HLC ratios at diagnosis and the role of HLC ratio in disease monitoring in 31 patients with Waldenström's macroglobulinemia. Median IgM HLC ratio was significantly higher in patients requiring treatment at presentation than in those not requiring treatment. IgM HLC ratio correlated with bone marrow infiltration and time to first treatment. A simple risk stratification model utilizing IgM HLC ratio > median, β_2M >5 mg/L and abnormal LDH identified 3 prognostic groups with respect to survival (p<0.001) in this series with a median follow – up of 59 months. Authors suggest that HLC IgM and IgM HLC ratio seem to separate patients with a more aggressive disease. Leleu et al. suggest that Hevylife test might replace the current techinque to measure IgM M-spike in the years to come [28].

IgG but not IgA HLC ratios have been shown to predict malignant transformation in MGUS patients [18].

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Table VI. Tumor and non-tumor HLC concentrations and HLC IgGκ/IgGλ, IgAκ/IgAλ, IgMκ/IgMλ ratios at diagnosis in sera of patients with IgG and IgA multiple myeloma and IgM malignant lymphoma with survival not exceeding 5 years

Tabela VI. Stężenia nowotworowej i nie nowotworowej HLC i wartości stosunku IgGκ/IgGλ, IgAκ/IgAλ, IgMκ/IgMλ HLC w chwili rozpoznania choroby w surowicach chorych na szpiczaka plazmocytowego IgG i IgA oraz chloniaka złośliwego IgM z czasem przeżycia poniżej 5 lat

Tumor sera with M-protein isotype acc. to IFE	n	HLC immunoglobulin g/L		HLC ratios IgGκ/IgGλ	n	HLC immunoglobulin g/L		HLC ratios IgAκ/IgAλ	n	HLC immunoglobulin g/L		HLC ratios IgMκ/IgMλ
		IgGκ x±SD Median, range	IgGλ x±SD Median, range	x±SD Median, range		IgAκ x±SD Median, range	IgAλ x±SD Median, range	x±SD Median, range		IgMκ x±SD Median, range	IgMλ x±SD Median, range	x±SD Median, range
MM IgGκ	6	27.97±12.68 30.65 8.5–40.4	2.17±3.35 0.854 0.47–9.00	37.31±27.7 43.29 2.14–73.79								
MM IgGλ	4	1.8±1.68 1.63 0.31–3.6	33.9±12.5 30.15 23.4–52.2	0.04±0.03 0.04 0.01–0.09								
MM IgAκ					4	41.4±38.0 29.9 10.5–83.9	0.12±0.02 0.11 0.10–0.15	338±336 189.00 102.94–742.47				
MM IgAλ					5	0.68±0.44 0.84 0.08–1.20	24.4±17.1 26.7 3.7–40.7	0.03±0.02 0.02 0.02–0.06				
ML IgMκ									17	66.28±48.77 52.9 10.90–144.0	0.29±0.36 0.23 0.008–1.58	831±1205 280 9.63–3610.00
ML IgMλ									7	0.53±0.52 0.31 0.04–1.58	45.0±33.1 37 16.7–106.0	0.01±0.01 0.01 0.01–0.05
												dla n=21 0.22±0.17 mediana 0.21

Table VII. Serum free light chain (FLC) concentrations at diagnosis in patients with multiple myeloma (MM) and IgM malignant lymphoma (ML) with survival exceeding 10 years

Tabela VII. Stężenia w surowicy wolnych łańcuchów lekkich (FLC) w chwili rozpoznania choroby u chorych na szpiczaka plazmocytowego i chłoniaka złośliwego IgM z czasem przeżycia ponad 10 lat

	n	κ FLC (mg/l)	λ FLC (mg/l)	κ/λ ratio
IgGκ MM Median; range	7	60.1; 46–532	11.6; 5.3–25.2	8.5; 2.3–99.8
IgGλ MM Median; range	6	10.0; 6.3–14.4	105.8 ;56.9–421	0.09; 0.03–0.12
IgAκ MM Median; range	7	24.3; 12.1–55.9	6.8; < 0.2–11.6	4.2; 1.4–46
IgAλ MM Median; range	2	6.7 6.4	26.3 71.7	0.26 0.09
IgMκ ML Median; range	9	79.5; 9.4–3450	11; 5.3–312	11.0; 2.0–28.8
IgMλ ML Median; range	3	12.3; 10.2–24.0	95.6; 39.4–123	0.13; 0.08–0.61
Healthy persons Median; range	10	13.5; 6.4–18.0	11.3; 6.6–23.3	1.0; 0.74–1.34

Table VIII. Serum free light chain (FLC) concentrations at diagnosis in patients with multiple myeloma and IgM malignant lymphoma with survival not exceeding 5 years

Tabela VIII. Stężenia w surowicy wolnych łańcuchów lekkich (FLC) w chwili rozpoznania choroby u chorych na szpiczaka plazmocytowego i chłoniaka złośliwego IgM z czasem przeżycia poniżej 5 lat

	n	K FLC (mg/l)	λ FLC (mg/)l	κ/λ ratio
IgGκ MM Median; range	6	877; 46–11400	4.8; 1.5–9.0	189; 5–7620
IgGλ MM Median, range	4	6.0; 0.07–22.8	3.1; 51.9–760	0.01; 0.001–0.08
IgAλ MM	2	2.02 undetectable	374 178	0.01 0.000…
IgMκ ML	1	3500	6.6	531
IgMλ ML	2	2.1; 5.8	409; 103	0.01; 0.06

Table IX. Comparison of serum immunoglobulin HLC ratios at diagnosis in MM patients with survival exceeding 10 years, not exceeding 5 years and patients enrolled to IFM 2005 trial.

Tabela IX. Porównanie wartości stosunku IgGκ/IgGλ, IgAκ/IgAλ HLC w surowicy w chwili rozpoznania choroby u chorych na szpiczaka plazmocytowego z czasem przeżycia ponad 10 lat, poniżej 5 lat i chorych włączonych do próby klinicznej IFM 2005

MM patients with M-protein isotype acc. to IFE	Present study		IFM 2005 trial (n=339) Avet-Loiseau et al. [16, 17] Bradwell et al. [24] involved/un-involved HLC ratio median, range
	Patients with survival >10 years (n=23) involved/un-involved HLC ratio median, range	Patients with survival <5 years (n=43) involved/un-involved HLC ratio median, range	
IgGκ MM	11.80 7.94–58.50	43.29 2.14–73	93.52 3.94–1334
IgGλ MM	0.09 0.06–0.35	0.04 0.01–0.09	0.018 0.001–1.05
IgAκ MM	20.90 5.13–79.30	189 102–742	462 8.8–7352
IgAλ MM	0.09; 0.16	0.02 0.02–0.06	0.01 0.001–0.32

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